



United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine



Trace Back Protocol
For Nurseries that Shipped Plant Material to a Confirmed
***Phytophthora ramorum* Infested Nursery**
28 September 2006
Version 1.1

Mission/Goal Statement

The goal is to find and eradicate the pathogen if present in nurseries. Any interpretation of this protocol that is contrary to this goal is a misinterpretation of the protocol. A detailed and thorough inspection should take place at the field level to identify the presence of *P. ramorum*. Areas of consideration are to include, but are not limited to, nursery stock, plant material and debris, soil, and water usage.

Purpose

The purpose of this protocol is to establish a set of procedures that are to be used to determine if a nursery that shipped plants to a *Phytophthora ramorum* confirmed positive nursery has infected plants in their inventory, and thus might be a potential source of *P. ramorum*, the plant pathogen that causes sudden oak death, ramorum blight, and ramorum die-back. By following the procedures in this protocol, we can ensure a consistent, science and risk based response to detections of *P. ramorum* in commercial nursery stock.

For more information on this pathogen please visit the USDA, APHIS, PPQ web site at:
<http://www.aphis.usda.gov/ppq/ispm/pramorum/>.

Definitions

- Associated Plants:** Associated plants are those reported found naturally infected and from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed (see Appendix A).
- Block:** Within a nursery, this is a contiguous block of Host and Associated Host Plants (HAP). The block will be considered contiguous until there is a 2 meter break of either no plants or no HAP.

Confirmed Positive:	The test result on a presumptive positive that <i>P. ramorum</i> is present based on DNA testing or culture morphology. This confirmation would be conducted by APHIS in the case of PASS samples or by the provisionally approved lab or diagnosticians with identification authority in the case of non-PASS samples.
Cull Pile:	An area where discarded plant material is deposited. Also known as a waste pile.
Destruction Block:	Block of plants to be destroyed. Within a nursery, this is a contiguous block of HAP containing one or more plants known to be infected with <i>P. ramorum</i> . The block will be considered contiguous until there is a 2 meter break of either no plants or no HAP.
Host And Associated Host Plants (HAP):	Host and associated host plants listed on the official “APHIS List of Regulated Hosts and Plants Associated with <i>Phytophthora ramorum</i> ”.
High Risk Plants:	These are any HAP which originated in the destruction block at the infested (source) nursery. These plants are to be identified using the best available information and to the lowest available taxonomy, (for example, if high risk plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level). All plants shipped from the infested nursery in the past 12 months which match the description of plants which were in the destruction block are considered to be high risk.
Infected Plants:	Plants officially confirmed as being infected with <i>P. ramorum</i> , based on the use of APHIS approved diagnostics.
Medium Risk Plants:	Any HAP found from an infested nursery which did not originate in the destruction block.
Nursery/Facility:	Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed. Locations that grow trees to be sold without roots (i.e. Christmas trees) and locations where such trees are stored or distributed are considered to be nurseries.
Quarantine Block:	Area identified as a 10 meter radius from the destruction block (see Appendix B) designed to determine if <i>P. ramorum</i> has spread beyond the destruction block. This zone is also known as the quarantine block. (Use of Quarantine Block is an adaptation from the definition: “An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production or a pest-free production site, and in which phytosanitary measures are taken to prevent spread of the pest.” [ISPM Pub. No. 10, 1999])

**PASS (Potentially
Actionable Suspect
Sample):**

A presumptive positive *P. ramorum* sample diagnosed or identified by a provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation. (For more information see: "PASS System Policy" at www.aphis.usda.gov/ppq/ispm/pramorum/protocols.html)

Suspected Infected Plants: These are plants with visible symptoms of *P. ramorum* infection; and/or HAP that are a part of an infested block or derived from an infested block or buffer zone; and/or plants that have tested positive using PCR or culturing, but have not been confirmed positive for *P. ramorum* by APHIS.

Trace Back (TB) Plants: All plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) regardless of size, location or lot, back to the original propagation source if still existing.

Trace Back (TB) Site: Any location that shipped potentially infected plants to a confirmed infested nursery, residence or commercial landscape.

Trace Forward (TF) Plants: Plants identified on a trace forward list as being potentially infected with *P. ramorum*.

Trace Forward (TF) Site: Any location that received potentially infected plants from a confirmed infested source nursery; including residential or commercial landscapes.

Before Inspection

1. An inspection must be conducted when favorable climatic conditions, pathogen infectivity, and host susceptibility occur at the same time. This is commonly in the spring or fall seasons. If conditions are not suitable for disease development at the time of detection, an additional inspection must be conducted when favorable climatic conditions are present.
2. For Federal inspectors, notify state officials of your plans to inspect.
3. Coordinate visit with State inspector.
4. Federal and State or County inspectors should contact the nursery owners/managers prior to the visit to determine how many plants of interest are still in stock and to arrange for the inspection. This nursery contact would normally occur within 24 hours of the expected arrival time for the TB inspection.
5. If you are unable to visit the nursery within one day of your contact with the nursery owner/manage, send a PPQ form 523, an Emergency Action Notification (EAN) by fax to them and request that they sign and return it to you by fax.

Survey/Inspection Procedures

1. Identify yourself and agency to the nursery/facility owner/manager.
2. Explain to the nursery/facility owner/manager the purpose of your visit.
3. Obtain copies of the shipping documents of all plants shipped from the same taxon (such as genus, species, hybrid, variety, or cultivar) to other nurseries or retail facilities within a thirty day period (pre and post) as the date that the trace back positive plant was shipped. This information is to be forwarded to SPHDs.
 - These documents are to track and survey these plants and to take advantage of when the fungicides would wear off naturally (and not normally reapplied by retail sites).
 - The identified nurseries should be considered when deciding priorities for the National Survey.
4. Determine the presence of any plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) that were reported at the trace forward site.
5. Ask owner/manager to fill out questionnaire (see Appendix C), or complete with their input.
6. **Sampling method:** Determining the number of plants to be inspected. The goal is targeted sampling of plant tissue to determine the presence of *P. ramorum* with a 95% confidence of finding the disease at a very low level (0.5% of plants are infected with *P. ramorum*) by inspecting a minimum of 850 TB HAP plants in each block (or all the plants if there are less than 850). A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. Do not sample asymptomatic plants. If TB HAP plants can not be determined, visually inspect at least 850 HAP plants of the same taxa from each block shipped from the TB nursery. If all TB plants have been sold or are no longer present, have the nursery personnel identify the growing area and then inspect any plants growing in that location.
7. Visually inspect the appropriate number of HAP for unhealthy tissue. To visually inspect a plant, carefully lift the plant from surrounding plants and examine all plant leaves and stems for unhealthy tissue particularly for but not limited to the presence of water-soaked or necrotic lesions consistent with *P. ramorum* infection. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms. Be sure to mark plant as visually inspected either with flagging with the appropriate sample number or a stake with the appropriate sample number. Also examine the leaves that have fallen off the plant for disease symptoms. A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. **Do not sample asymptomatic plants**, but feel free to sample any and all plants exhibiting unusual or atypical plant tissue. Images of *P. ramorum* symptoms are available at <http://www.aphis.usda.gov/ppq/ispm/pramorur/> . Keep in mind that these images should only be used as a reference, and should not be considered all inclusive for each species. Links to other sites such as <http://www.suddenoakdeath.org> , which provide nursery guides that describe and illustrate *P. ramorum* infections are also available at <http://www.aphis.usda.gov/ppq/ispm/pramorur/> . These nursery guides may be viewed and printed from these sites. Symptoms of *P. ramorum* may include:

- Leaf spots
- Twig dieback
- Stem cankers

Keep in mind that many other pathogens cause similar symptoms. Remember that other symptoms caused by *Phytophthora ramorum* as yet unseen may be detected, so sample any unusual or atypical plant symptoms.

8. Sample plant tissue from any and all visually inspected plants that appear unhealthy. Each sample should consist of a minimum of five leaves; for vaccinium and other small leaf hosts collect the terminal last 3 inches of branch tips, if present, from each unhealthy plant. If, however, only one leaf is symptomatic include only the one leaf with lesions.

For all samples:

- Fill out PPQ Form 391 (Name of host, variety, state code, facility code, etc.).
 - Assign a unique sample number using the following conventions: XX-ABC-0001 where XX is your two-letter state code, ABC is a three-letter, state-assigned facility code, and 0001 is the sample number for that facility.
 - Log each sample according to the unique sample number.
 - Double bag samples (e.g., symptomatic leaf tissue with associated twig intact) in plastic bags.
 - Label with collection date, time, location, responsible party. Be sure to write sample number on the bag containing the sample.
 - Be sure to mark sampled plants either with flagging with the appropriate sample number or a stake with the appropriate sample number.
 - Cool, but do not freeze specimen.
 - Submit with minimal delay to your designated laboratory for analysis. All tissue samples must be analyzed by APHIS-approved or APHIS provisionally-approved Laboratory using the appropriate diagnostic protocols.
 - Overnight the sample if necessary – do not send samples on Friday or the day before lab holidays – check with the lab – as they may not be received until the following Monday. Samples stored without refrigeration may deteriorate and not be testable. Identify the sample(s) as Trace Back (TB) Sample to distinguish the sample(s) from National Survey samples.
9. Ask owner/manager to identify cull piles. Check cull piles for *P. ramorum* symptomatic plants and plant material. Sample as above, if observed.
 - Include appropriate sampling and testing of soil and water. Soil should be tested underneath cull piles. Water should be baited and sampled from downward slopes, as well as any collected or drained reservoirs from the site.
 10. If the survey requires the inspector to move among multiple greenhouses, shade houses or discrete blocks, disinfect tools, hands and shoes (or wear disposable gloves and tyvek booties) to prevent pathogen spread between areas. If using disposable gloves and booties, be sure to dispose of these after each individual greenhouse/shade house/block inspection. Disposable rubber gloves and tyvek booties can also be disinfected using 10% bleach solution or a quaternary ammonium solution (at the labeled rate) between inspecting each area. See Exhibit B for details on disinfectants and fumigants for use in nurseries. (Washtubs with ~ 1/2 inch of disinfectant to step in for booties and 3 inches in buckets to dip gloved

hands should be sufficient.) Be sure to properly disinfect booties and gloves between all nursery blocks. Disposable gloves and booties should be bagged and disposed by burial or incineration, or in a landfill upon completion of inspections.

11. Complete an Emergency Action Notification (EAN, PPQ form 523) or State equivalent, to place a hold on all blocks containing symptomatic plants from the trace back investigation until lab results are released.
12. Sanitize/disinfest tools and shoes before leaving premises, using an appropriate disinfectant for the control of *Phytophthora spp.* (such as a 10% solution of bleach or quaternary ammonium solution at labeled rates). See Exhibit D for details on disinfectants and fumigants for use in nurseries.

Laboratory Results/Regulatory Response

1. If plant samples are found positive for *P. ramorum* whether the plants are remaining on the site or have been destroyed, the APHIS, PPQ Confirmed Nursery Protocol will be applied.
2. If samples associated with the cull piles are found positive for *P. ramorum*, the APHIS, PPQ Confirmed Nursery Protocol will be applied.
3. If samples of water or soil are found positive for *P. ramorum*, the Regional Program Manager will provide guidance on proper response.
4. If samples are found negative for *P. ramorum*, thank the owner/manager of the nursery for their cooperation and release the EAN.

Appendix A

APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*

(Revision dated 11 September 2006)

This list is continually being updated.

The most current version is posted at: <http://www.aphis.usda.gov/ppq/ispm/pramorum>

Proven Hosts Regulated for *Phytophthora ramorum*

Scientific Name (47)	Common Name(s)	Notes
<i>Acer macrophyllum</i>	Bigleaf maple	
<i>Acer pseudoplatanus</i>	Planetree maple	Koch's postulates completed
<i>Aesculus hippocastanum</i>	Horse chestnut	Koch's postulates completed
<i>Adiantum aleuticum</i>	Western maidenhair fern	
<i>Adiantum jordanii</i>	California maidenhair fern	
<i>Aesculus californica</i>	California buckeye	
<i>Arbutus menziesii</i>	Madrone	
<i>Arctostaphylos manzanita</i>	Manzanita	
<i>Calluna vulgaris</i>	Scotch heather	
<i>Camellia</i> spp.	Camellia - all species, hybrids and cultivars	
<i>Castanea sativa</i>	Sweet chestnut	
<i>Fagus sylvatica</i>	European beech	
<i>Frangula californica</i> (≡ <i>Rhamnus californica</i>)	California coffeeberry	
<i>Frangula purshiana</i> (≡ <i>Rhamnus purshiana</i>)	Cascara	
<i>Fraxinus excelsior</i>	European ash	
<i>Griselinia littoralis</i>	Griselinia	

<i>Hamamelis virginiana</i>	Witch hazel	
<i>Heteromeles arbutifolia</i>	Toyon	
<i>Kalmia latifolia</i>	Mountain laurel	
<i>Lithocarpus densiflorus</i>	Tanoak	
<i>Lonicera hispidula</i>	California honeysuckle	
<i>Laurus nobilis</i>	Bay laurel	Koch's postulates completed
<i>Maianthemum racemosum</i> (\equiv <i>Smilacina racemosa</i>)	False Solomon's seal	
<i>Michelia doltsopa</i>	Michelia	Koch's postulates completed
<i>Parrotia persica</i>	Persian ironwood	
<i>Photinia fraseri</i>	Red tip photinia	
<i>Pieris floribunda</i> and <i>Pieris floribunda</i> \times <i>japonica</i> & all hybrids of <i>P. floribunda</i>	Mountain Andromeda	
<i>Pieris formosa</i> and <i>P. formosa</i> \times <i>japonica</i> & all hybrids of <i>P. formosa</i>	Himalaya Andromeda	
<i>Pieris japonica</i> & all hybrids of <i>P. japonica</i>	Japanese Pieris	
<i>Pseudotsuga menziesii</i> var. <i>menziesii</i> & all nursery grown <i>P. menziesii</i>	Douglas fir	
<i>Quercus agrifolia</i>	Coast live oak	
<i>Quercus chrysolepis</i>	Canyon live oak	
<i>Quercus cerris</i>	European turkey oak	
<i>Quercus falcata</i>	Southern red oak	
<i>Quercus ilex</i>	Holm oak	

<i>Quercus kelloggii</i>	California black oak	
<i>Quercus parvula</i> var. <i>shrevei</i> & all nursery grown <i>Q. parvula</i>	Shreve's oak	
<i>Rhododendron</i> spp.	Rhododendron (including azalea) – all species, hybrids and cultivars	
<i>Rosa gymnocarpa</i>	Wood rose	
<i>Salix caprea</i>	Goat willow	
<i>Sequoia sempervirens</i>	Coast redwood	
<i>Syringa vulgaris</i>	Lilac	
<i>Taxus baccata</i>	European yew	
<i>Trientalis latifolia</i>	Western starflower	
<i>Umbellularia californica</i>	California bay laurel, pepperwood, Oregon myrtle	
<i>Vaccinium ovatum</i>	Evergreen huckleberry	
<i>Viburnum</i> spp.	Viburnum – all species, hybrids and cultivars	

Plants Associated with *Phytophthora ramorum*

(These are regulated only as nursery stock)

Scientific Name (58)	Common Name, Date & Source of Report	Notes
<i>Abies concolor</i>	White fir – Oct 05 (1)	
<i>Abies grandis</i>	Grand fir – June 03 (1)	
<i>Abies magnifica</i>	Red fir – Jan 06 (7)	
<i>Acer circinatum</i>	Vine maple – Feb 06 (5)	
<i>Acer davidii</i>	Striped bark maple – Jan 06 (9)	
<i>Acer laevigatum</i>	Evergreen Maple – Aug 05 (3)	
<i>Arbutus unedo</i>	Strawberry tree – Dec 02 (7)	
<i>Arctostaphylos columbiana</i>	Manzanita – Feb 06 (5)	
<i>Ardisia japonica</i>	Ardisia – Jan 06 (9)	
<i>Calycanthus occidentalis</i>	Spicebush – May 05 (5)	
<i>Castanopsis orthacantha</i>	Castanopsis - Aug 06 (3)	New listing - Reported found in the UK
<i>Ceanothus thyrsiflorus</i>	Blueblossom – April 06 (5)	
<i>Cinnamomum camphora</i>	Camphor tree – May 06 (3)	
<i>Clintonia andrewsiana</i>	Andrew's clintonia bead lily – May 04 (5)	
<i>Cornus kousa x Cornus capitata</i>	Cornus Norman Haddon – Aug 06 (3)	New listing - Reported found in the UK
<i>Corylus cornuta</i>	California hazelnut – Dec 02 (5)	
<i>Distylium myricoides</i>	Myrtle-leaved Distylium – Jul 06 (9)	New listing - Reported found in Canada
<i>Drimys winteri</i>	Winter's bark – July 04 (3)	

<i>Dryopteris arguta</i>	California wood fern – May 04 (5)	
<i>Eucalyptus haemastoma</i>	Scribbly gum – Aug 06 (3)	New listing - Reported found in the UK
<i>Euonymus kiautschovicus</i>	Spreading euonymus – Jan 06 (9)	
<i>Fraxinus latifolia</i>	Oregon ash – Aug 05 (5)	
<i>Gaultheria shallon</i>	Salal, Oregon wintergreen – Jan 06 (9)	
<i>Hamamelis x intermedia</i> (<i>H. mollis</i> & <i>H. japonica</i>)	Hybrid witchhazel – Jan 06 (9)	
<i>Hamamelis mollis</i>	Chinese witchhazel – Jan 05 (3)	
<i>Ilex purpurea</i>	Oriental holly – Jul 06 (9)	New listing - Reported found in Canada
<i>Kalmia angustifolia</i>	Sheep laurel – May 06 (3)	
<i>Leucothoe axillaris</i>	Fetterbush, dog hobble – Jan 06 (9)	
<i>Leucothoe fontanesiana</i>	Drooping leucothoe - Oct 03 (3)	
<i>Loropetalum chinense</i>	Loropetalum – Jul 06 (9)	New listing - Reported found in Canada
<i>Manglietia insignis</i>	Red lotus tree – Aug 06 (9)	New listing - Reported found in Canada
<i>Magnolia grandiflora</i>	Southern magnolia – Jan 06 (9)	
<i>Magnolia stellata</i>	Star magnolia – Jan 05 (3)	
<i>Magnolia x loebneri</i>	Loebner magnolia – Jan 05 (3)	
<i>Magnolia x soulangeana</i>	Saucer magnolia – Jan 05 (3)	
<i>Michelia maudiae</i>	Michelia – Jan 06 (9)	
<i>Michelia wilsonii</i>	Michelia – Jan 06 (9)	
<i>Nerium oleander</i>	Oleander – June 06 (1)	

<i>Nothofagus obliqua</i>	Roble beech – Dec 04 (3)	
<i>Osmorhiza berteroi</i>	Sweet Cicely – Aug 05 (5)	
<i>Osmanthus decorus</i> (≡ <i>Phillyrea decora</i> ; ≡ <i>P. vilmoriniana</i>)	Osmanthus – Jan 06 (9)	
<i>Osmanthus fragrans</i>	Sweet olive – June 06 (1)	
<i>Osmanthus heterophyllus</i>	Holly olive – June 06 (1)	
<i>Parakmeria lotungensis</i>	Eastern joy lotus tree – Jul 06 (9)	New listing - Reported found in Canada
<i>Pittosporum undulatum</i>	Victorian box – Dec 02 (6)	
<i>Prunus lusitanica</i>	Portuguese laurel cherry – Jan 06 (9)	
<i>Pyracantha koidzumii</i>	Formosa firethorn – Apr 04 (9)	
<i>Quercus acuta</i>	Japanese evergreen oak – May 06 (3)	
<i>Quercus petraea</i>	Sessile oak – Aug 05 (3)	
<i>Quercus rubra</i>	Northern red oak – Nov 03 (8)	
<i>Rosa</i> (specific cultivars) Royal Bonica (tagged: “MEImodac”) Pink Meidiland (tagged: “MEIpoque”) Pink Sevillana (tagged: “MEIgeroka”)	Hybrid roses – Jan 06 (9)	Revised listing - Note that these are specific registered cultivars which can be identified by the listed tags
<i>Rosa rugosa</i>	Rugosa rose – Jan 06 (9)	
<i>Rubus spectabilis</i>	Salmonberry – Dec 02 (4)	
<i>Taxus brevifolia</i>	Pacific yew – May 03 (5)	
<i>Taxus x media</i>	Yew – June 05 (8)	
<i>Torreya californica</i>	California nutmeg – Aug 05 (5)	

<i>Toxicodendron diversilobum</i>	Poison oak – Dec 02 (4)	
<i>Vancouveria planipetala</i>	Redwood ivy – Aug05 (5)	

- ¹ California Department of Food and Agriculture, Sacramento, CA
- ² Oregon Department of Agriculture. Salem, OR
- ³ Department for Environment, Food and Rural Affairs, UK
- ⁴ Everett Hanson, Oregon State University, Corvallis, OR
- ⁵ David Rizzo, University of California, Davis, CA
- ⁶ Matteo Garbelotto, University of California, Berkeley, CA
- ⁷ Gary Chastagner, Washington State University, Puyallup, WA
- ⁸ Plant Protection Service, Wageningen, Netherlands
- ⁹ Canadian Food Inspection Agency, Ottawa, Ontario, Canada
- ¹⁰ (Reserved)
- ¹¹ (Reserved)

Rationale for Lists:

Host Plants Regulated for *Phytophthora ramorum*:

Naturally infected associated plants are deemed host plants regulated for *P. ramorum* upon completion, documentation, review and acceptance of traditional Koch's postulates. Details on regulated plants and articles can be found via links to "Phytophthora ramorum 7 CFR 301.92" and "Recent Modifications to Phytophthora ramorum Regulations" at:

<http://www.aphis.usda.gov/ppq/ispm/pramorum>

The plants listed in the original Interim Rule dated 14 February 2002 were adapted from a review and evaluation of lists of regulated plants from other regulatory agencies.

Plants Associated with *Phytophthora ramorum*:

Plants associated with *P. ramorum* are naturally infected plants and from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). Traditional Koch's postulates have not yet been completed nor documented and reviewed for each of these associated plants. These reports must be documented and reviewed by PPQ before they will be listed.

Regulation at the genus level:

Plants included in either of the above lists may be regulated at the genus level. This will ensure appropriate and effective inspection in quarantine areas, regulated nurseries, and regulated articles to mitigate the spread of *P. ramorum*. An example is when the number of individual species, hybrids, or cultivars listed or to be listed is determined to hinder appropriate and effective inspection or regulation.

Agency Contact:

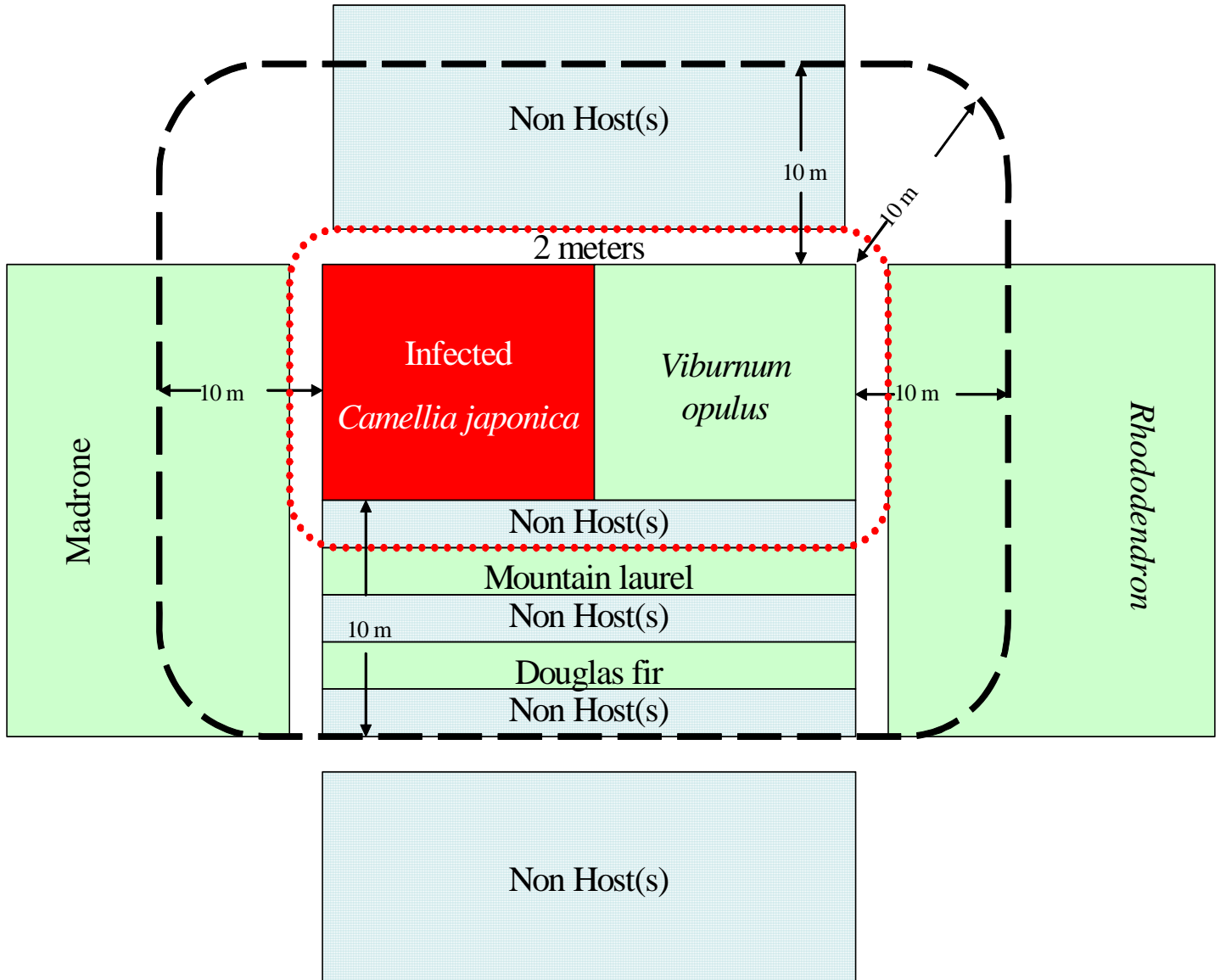
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Appendix B

Schematic of Nursery with Infected Host Plant(s)



Destruction Block

Action: Destroy *Camellia japonica* and *Viburnum opulus*. Hold and monitor all non-hosts.



Quarantine Block

Action: Hold and monitor all Mountain laurel and Douglas fir, as well as some Madrone and Rhododendron.

Appendix C

Phytophthora ramorum Questionnaire (Property Owner or Manager): Part 1

Name of Nursery: _____

Name of Owner or Manager: _____

Address of Site: _____

City: _____, State: _____, Zip Code: _____

Contact Name: _____, Title: _____

Phone Number: _____, Fax Number: _____

GIS Coordinates (if available): _____

Type of Facility (circle): Nursery Greenhouse Wholesale
Other _____

1. Are you the owner or manager of the property or facility? If not please provide owner contact information. _____
2. Did you purchase the plant(s) in question? (If “no”, seek information on individual who planted material in question). _____
3. How long ago did you purchase the plant(s)? _____
4. Did you purchase any other plants from this same nursery? _____
5. Have you noticed any other problems with plants on your property? _____
6. Have you moved any plants, received from the infested nursery, from your primary retail location to a different location? _____
 - a) What types and varieties (cultivars) were they?

 - b) How long ago was that? _____
 - c) What is the address of that location? _____

7. Did you move any plants here from a different location? _____
- a) What types and varieties were they? _____

- b) How long ago was that? _____
- c) What is the address of that location? _____
8. Do you have a landscape company that purchases plants from you? _____
9. What is the contact information for the landscape company? _____
10. What is your source of water? _____

***Phytophthora ramorum* Questionnaire (Property Owner or Manager): Part 2**

Information on suspect plant material for inspector visiting property:

1. What is the variety and number of plants? _____
2. What is the condition of the plant material? _____
3. Have the plants been trimmed or pruned? _____
4. How are the trimmings disposed of? _____
5. Did the plant material come in pots? _____
 - a) Did you dispose of the pots or re-use them? _____
6. If the pots were reused or stored, describe how the pots were handled?

Appendix D

Treatment and Disinfection

The following techniques are approved by USDA APHIS PPQ for control of *P. ramorum* in nurseries found to contain plants infected with *P. ramorum*.

Infected Plants:

Note: HAP material, including leaf litter, must not be placed in compost piles or be removed from the nursery site as trash or in debris removal. HAP material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.

- **Incineration (burning to ash):** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.
- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.
- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for “insect pests and pathogens in garbage”, Schedule T415b (http://www.aphis.usda.gov/ppq/manuals/pdf_files/Treatment%20Chapters/05-05-T400-5.pdf).

Non-Porous Surfaces:

Most disinfectants are not labeled for use in soil and are only useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is **practical**, tools such as knives, pruners, water breakers, water wands and other implements used in the buffer area should only be used in the buffer area. If tools and other implements must be moved from the buffer area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the buffer zone. The following table modified from <http://cpmcnet.columbia.edu/dept/ehs/decon.html> examines the effects of different classes of disinfectants on microbial populations. This list is for explanation and information only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

Disinfectant	Trade names	Comments	Contact time
Alcohols (ethyl and isopropyl) 60-85%	Lysol Spray	Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable.	10-15 minutes
Phenolics (0.4%-5%)	Pheno-cen	Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue.	10-15 minutes
Quaternary Ammonium (0.5-1.5%)	Consan Triple Action 20 Physan 20 Green-Shield 20	Effective for non-porous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels.	10-15 minutes
Chlorine (100-1,000 ppm)	10% Clorox 10% Bleach	Inactivated by organic matter; fresh solutions of hypochlorite (Clorox) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. Exposure to sunlight further reduces hypochlorite efficacy. Keep solution in opaque container.	10-15 minutes

Water:

- **For dust abatement, fire suppression, and equipment cleaning:** Clorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning. The active ingredient level must be measured from water collected at the sprinkler head.
- **For irrigation:** Chlorine levels of 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, recirculated, non-municipal water, must be chlorinated at an active chlorine concentration equal to or greater than 2 mg/liter of water; for facilities that recycle water, this chlorine level must be monitored.

Soil and Potting Media:

- **Potting media:** Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- **Soil:** Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks.

All fumigants are restricted use and must be applied according to labels by a licensed applicator. Any use of pesticides in any manner not listed on the label is unlawful.

Summary of Labeled Soil Fumigants

Fumigant	Trade names	Comments
Chloropicrin	Chlor-O-Pic Metapicrin Timberfume Tri-Clor	Often used in combination with methyl bromide due to its ability to be detected in small quantities.
Dazomet	Basamid	Methyl isothiocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated.
Metam-sodium	Busan 1020 Busan 1180 Busan 1236 Metam Vapam	Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling.
Methyl Bromide	Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom	Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential.

Physical Treatment of Soil:

- Mitigation of infested soil can also be achieved by installing permanent impermeable, non-porous barriers that consist of cement, concrete or asphalt. These barriers must be constructed so that no native soil within the destruction block is visible. The barriers should be graded such that no standing water can be observed.

Equipment and Personnel (Inspectors and employees):

- Access to infested areas and hold areas should be limited, as much as possible, to officials and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- Personnel should not have access to other parts of the nursery after entering the destruction block on the same day.
- A disinfectant foot bath should be placed and used by personnel entering and exiting the buffer zone and entering and exiting the destruction block at the infested nursery site, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with filth, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the buffer zone or destruction block. The disposable shoe covers must be placed in bags and incinerated or deep-buried.
- The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.
- Do not visit other nursery sites in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the nursery, the nursery should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris by the visitor.
- Wood surfaces suspected of contamination with *P. ramorum* should be disposed of as stated above under "Infected Plants".